United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	Fertility Assessments in SP22 KO mice
LAPR Number:	18-05-003
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6/RTP/USEPA/US
Date Originated:	05/07/2015
LAPR Expiration Date:	05/31/2018
Agenda Date:	05/20/2015
Date Approved:	06/01/2015
Date Closed:	04/12/2018

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	05/29/2015	DMR	
	Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	06/01/2015	DMR	

Administrative Information

1. Project Title (no abbreviations, include species):
Fertility Assessments in SP22 KO mice

Is this a continuing study with a previously approved LAPR?
Yes
Please provide the previous LAPR# 15-05-002

2. Programatic Information
a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.
CSS 12.01 AOP Discovery and Development.
b. What is the Quality Assurance Project Plan (QAPP) covering this project?
IRB-NHEERL-RTD/GEEBB/Exemption Exemption Exemption-FY03-001-000

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6 *Lotus Notes Address* Exemption Exemption Exemption Exemption 6/RTP/USEPA/US	TAD *Branch* RTB	MD

4. Alternate Contact:

Alternate Contact	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6 *Lotus Notes Address* Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US	TAD *Branch* RTB	MD

SECTION A - Description of Project

1. Explain the study objective(s) in <u>non-technical language</u> such that it is understandable by non-scientific

persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

A biomarker that can accurately detect the fertility of human sperm in an epidemiology study is pivotal to addressing the current belief that human exposure to environmental chemicals is decreasing the quality of human semen. Our studies have shown sperm protein SP22 to be essential for fertility; we are working to develop SP22 as a potential biomarker in human epidemiology/fertility assessments. In our previous LAPR (15-05-002), we have been using SP22 knockout (KO) mice with to determine how fertility might be altered in an animal in which the SP22 protein is not expressed. Initial breeding studies using SP22 intact and knockout mice indicated that both males and females not expressing SP22 have significantly reduced fertility. We have extended the initial study to a study in which knockout mice were compared using both CD-1 and BL6 wild type backgrounds. This follow-up breeding study is in progress and will be completed in this proposed LAPR. The purpose of this LAPR is just to complete the breeding study that was started under the expiring LAPR.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

Whole mice are needed to perform in vivo breeding studies. Mature gametes are terminally differentiated cells that cannot be generated in vitro at this time but must be produced by whole animals (mature females and adult males). We have conducted a PubMed search to confirm that reliable methods for growing or generating mature sperm and oocytes in vitro do not exist.

b. Justify the species requested:

The mouse is selected as an animal model for it is the only SP22 knockout model available.

3. How was it determined that this study is not unnecessary duplication?

A PubMed search confirmed that these studies have not been done previously. No study has evaluated the reproductive competence in male or female SP22 knockout mice. Search terms used were PARK7, DJ-1,SP22, reproductive, targeted mutation and knock out. (The SP22 protein is also known as PARK7 and DJ-1.)

SECTION B - In Vivo Procedures

1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

Matings with mice having a BL6 background were completed in the expiring LAPR. For this there were four groups of matings:

1) 6 wild type (WT) males x 6 WT females
2) 6 WT males x 6 KO females
3) 6 KO males x 12 WT females [6 bred one week, 6 the next next week]
4) 6 KO males x 6 KO females

Matings of KO mice with CD-1 KO mice will take place under the new LAPR. For this there will be only two groups of matings:

1) 6 CD-1 WT males x 6 KO females
2) 6 KO males x 12 CD-1 WT females [6 bred one week, 6 the next next week].

Litters will be examined for number, weight, and sex of pups.

Upon euthanasia and necropsy of males, epididymal sperm may be extracted for measurement of sperm counts and sperm motility. Additionally, sperm fertility may be assessed in vitro using ova obtained from WT females in a different LAPR (18-05-002).

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

Sixty adult animals and approximately 35 pups will be transferred from the expiring LAPR:
BL6 WT: 12 males, 6 females
BL6 KO: 12 males, 12 females
CD1 WT: 6 males, 12 females
Five litters x approximately 7 pups/litter = 35 offspring

Based on our previous data we expect roughly 200 offspring to be born from CD-1 x KO matings.

Total adults: 60 transferred
Total offspring: 35 transferred + 200 born = 235

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	60	235
D) Potential pain/distress relieved by appropriate measures:		
E) Unrelieved pain/distress:		

4. Does this LAPR include any of the following:

☐ Restraint (>15 Minutes) ☐ Survival surgery
☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):
None
b. Survival Blood Collections (method, volume, frequency):
N/A
c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):
N/A
d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:
N/A
e. Breeding for experimental purposes (e.g. length of pairing, number of generations):
Males and females are cohabitated for one week
f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:
Parental males and females have been ear-tagged. Adults are weighed at least weekly. Post-breeding, the potentially pregnant females are weighed twice per week for the first 2 weeks post-breeding, and then 5 days/week until parturition.

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):
N/A
b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):
N/A
c. Testing methods:
N/A
d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for

assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):
N/A
e. Describe how animals will be monitored (e.g., frequency of observations, by whom):
N/A
f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:
N/A
g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:
N/A

7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:
N/A
b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:
N/A
c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):
N/A
d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):
N/A
e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?
◯ Yes ◯ No
f. Identify any surgical procedures performed at other institutions or by vendors:
N/A

8. Humane interventions (for treatments/procedures in all categories).

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.
No deleterious effects are expected from the breeding protocol. Issues concerning animal health will be referred to the veterinarian. Sick or unhealthy animals will be euthanized.

Any mice maintained past 11 months of age will be monitored more frequently with particular attention to neurological effects (e.g., seizures) and dermatitis that have been seen in a previous colony of aged SP22 KO mice.

b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.
If animals (dams or pups) show symptoms of physical injury, dystocia, deteriorating body condition, lethargy, shivering, isolation of lone mice with ruffled fur, arched back, unstable movement, seizures, dermatitis, lack of interest in food, water, or social interaction we will euthanize or otherwise follow AV recommendations.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study:

b. Animals to be transferred from another LAPR: 95

LAPR Number that is the source of this transfer: 15-05-002

c. Animals to be transferred from another source:

d. Offspring produced onsite (used for data collection and/or weaned): 200

e. TOTAL NUMBER of animals for duration of the LAPR 295

2. Species (limited to one per LAPR): Mouse/Mice

3. Strain: C57BL/6 mouse/mice and CD1

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

N/A

4. Sources of animals:

Jackson Laboratories (C57BL/6) and Charles River (CD1)

5. Provide room numbers where various procedures will be performed on animals:

Exemption 6

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

N/A *Room Numbers:*

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

N/A

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

N/A

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

N/A

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

Prior to breeding, the female mice are housed 3/cage on pine shavings in a solid bottom cage with red igloos. During breeding, one female is housed with one male. After breeding, females are housed one per cage with red igloos.

Animals currently housed singly under the expiring LAPR will be transferred to this LAPR and remain singly housed with pine shavings and red igloos. Any new males will be socially housed until breeding or until they show aggression. They will then be housed 1 per cage with igloos.

KO mice maintained after 11 months of age will be provided alpha-dri for bedding.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

No agents will be administered.

2. Describe compounds to be administered to animals.

a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.

N/A

b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.

N/A

c. Provide a statement regarding any safety precautions necessary for handling any of these materials.

N/A

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING

Exemption 6	Principal Investigator	Supervise	Completed all NHEERL-required training. Has 36 years experience in handling rodents.
Exemption 6	Technical Staff	Set-up experiments,	Completed all NHEERL-required training. Has 38 years experience in handling rodents.
Exemption 6	Technical Staff	Set-up experiments, sacrifice mice to retrieve gametes.	Completed all NHEERL-required training. Has 28 years experience in handling rodents.
Exemption 6	Technical Staff	Breeding	Completed all NHEERL-required training Has 32 years experience handling rodents
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year

2. Breeding protocols and recordkeeping

3. Methods for monitoring genetic stability

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

Parental males will be euthanized after breeding is complete. Females will be euthanized after breeding is complete and pups have been euthanized. Pups will be killed by decapitation on or before postnatal day 14 using a sharp pair of scissors. (Backup scissors will be available.)

Except for KO animals, all mice will be euthanized by the end of August 2015. Because of their genetic value, KO mice may be maintained longer. Any mice maintained after 11 months of age will be housed on alpha-dri bedding and will be monitored more frequently.

2. Describe the euthanasia techniques:

Method(s): Anesthesia plus cervical dislocation
Agent(s): Sodium pentobarbital
Dose (mg/kg): 200 mg/kg
Volume: 0.1 ml/gm of 20 mg/ml
Route: ip

Source(s) of information used to select the above agents/methods:

_ 2013 AVMA Guidelines on Euthanasia.

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing

more than 200 grams).
N/A

4. Describe how death is to be confirmed.
Vital organ section

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.
Euthanized by Animal Care Contractor
The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?
● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	05/07/2015

Submitted: 05/18/2015

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	*Approval Date*	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	05/18/2015 by Exemption 6	Exemption 6	TAD	MD
		Lotus Notes Address	*Branch*	*Submitted to Branch Chief for Approval*
		Exemption 6	DTB	05/18/2015 09:35 AM

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ATTACHMENTS

Actions

First Update notification sent: 04/13/2016
Second Update notification sent:
First 2nd Annual notification sent: 04/03/2017
Second 2nd Annual notification sent:

1st Expiration notification sent: 04/03/2018
2nd Expiration notification sent:

History Log: